# A New Method of in Situ Activation for a Novel Class of DNA Alkylating Agents: Tunable Metal Cation Complexation and Activation

Dale L. Boger,* Scott E. Wolkenberg, and Christopher W. Boyce

*Department of Chemistry and Skaggs Institute for Chemical Biology The Scripps Research Institute, 10550 North Torrey Pines Road La Jolla, California 92037*

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Several well-established methods have been discovered or developed for selective activation of DNA binding agents which initiate reactivity toward DNA including reductive activation (mitomycins), oxidative activation (aflatoxin), disulfide or trisulfide cleavage (calicheamicin), photochemical activation (psoralen), and oxidant activation of metal complexes (bleomycin).[1,2] Many of these have been exploited in the development of therapeutics to impart selective activity against tumor cells or to provide a means to effect and control reactivity toward DNA for use as research tools (e.g., synthetic nucleases). Herein, we report a new means of in situ activation for a novel class of DNA alkylating agents. Methyl 1,2,9,9a-tetrahydrocyclopropa[*c*]pyrido[3,2-*e*]indol-4-one-7-carboxylate (CPyI, **1**) contains a unique 8-ketoquinoline structure which provides a tunable means to effect activation via selective metal cation complexation (Figure 1). This activation promotes a DNA minor groove adenine N3 alkylation in a manner analogous to that of CC-1065 and the duocarmycins, upon which CPyI was based.[3]

A study[3] of CPyI activation by metal cations toward nucleophilic addition (MeOH, Table 1) conducted in conjunction with its characterization revealed that the relative reaction rates correspond beautifully to the established stabilities of the resulting metal complexes (8-hydroxyquinoline, $Cu^{2+}$ > $Ni^{2+}$ > $Zn^{2+}$ > $Mn^{2+}$ > $Mg^{2+}$).[4] This provides the opportunity to predict, control, and tune the reactivity over a wide range depending on the application and conditions. Notably, $Mg^{2+}$ provided a rate which was not distinguishable from a background rate of BOC methanolysis, indicating that this prominent endogenous metal cation, like $Na^+$,[3] does not appear to activate CPyI effectively.

Consistent with this behavior, the efficiency of the DNA alkylation reaction of **1** (w794 DNA),[5] which occurs at $10^{-2}$ M for **1**−**3** (24 h, 25 °C for **1**, 37 °C for **2** and **3**), was dramatically increased in the presence of $Cu(acac)_2$ (100×), $Ni(acac)_2$ (100−1000×), and $Zn(acac)_2$ (1000×), the three metals selected for study, Table 2. This enhancement increased with increasing metal cation concentration for **1**, but not **2** and **3**, and resulted in no change in the DNA alkylation selectivity of **1**, which was identical to **2** and **3**. Typically studies were conducted with 1, 10, 100, and 1000 equiv of the metal cation with the former producing significant effects and the latter two concentrations providing the maximal effects. Under the conditions of our study, the enhancement was especially remarkable with $Zn(acac)_2$ (1000×) which promoted DNA alkylation of **1** at $10^{-5}$ M.[6] Alkylation at such low concentrations is unprecedented for such simple alkylation subunits, and this efficiency is within 10-fold of the natural

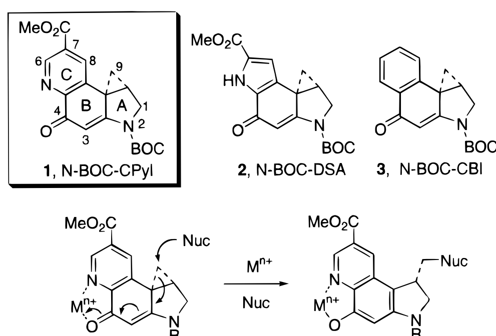


**Figure 1.**

**Table 1.** Metal-Catalyzed Addition to *N*-BOC-CPyI[a]

| metal | equiv | $k_{obs}$ ($s^{-1}$) | $t_{1/2}$ (h) |
|---|---|---|---|
| $Cu(acac)_2$ | 1.0 | $5.57 \times 10^{-5}$ | 3.5 |
| $Ni(acac)_2$ | 1.0 | $3.51 \times 10^{-5}$ | 5.5 |
| $Zn(acac)_2$ | 1.0 | $1.67 \times 10^{-5}$ | 11.5 |
| $Cr(acac)_3$ | 1.0 | $9.73 \times 10^{-6}$ | 20 |
| $Fe(acac)_3$ | 1.0 | $4.09 \times 10^{-6}$ | 47 |
| $Mn(acac)_2$ | 1.0 | $9.79 \times 10^{-7}$ | 200 |
| $Mg(acac)_2$ | 1.0 | $<7 \times 10^{-7}$ | >250 |
| none | none | stable | stable |

[a] Reaction run in $CH_3OH$ (25 °C) with addition of $CH_3OH$ to least substituted carbon of cyclopropane (regioselectivity >40:1).

**Table 2.** Metal Cation Effect on the DNA Alkylation Efficiency of **1**[a]

| metal | equiv | alkylation conc (M) | efficiency enhancement |
|---|---|---|---|
| none | none | $10^{-2}$ | − |
| $Cu(acac)_2$ | 1 | $10^{-2}$−$10^{-3}$ | 5× |
| $Cu(acac)_2$ | 10 | $10^{-3}$ | 10× |
| $Cu(acac)_2$ | 1000 | $10^{-4}$ (weak) | 10−100× |
| $Ni(acac)_2$ | 10 | $10^{-3}$ | 10× |
| $Ni(acac)_2$ | 1000 | $10^{-4}$ | 100× |
| $Ni(acac)_2$ | 10000 | $10^{-5}$ (weak) | 100−1000× |
| $Zn(acac)_2$ | 1 | $10^{-3}$ | 10× |
| $Zn(acac)_2$ | 10 | $10^{-4}$ | 100× |
| $Zn(acac)_2$ | 1000 | $10^{-5}$ | 1000× |

[a] w794, lowest concentration of **1** at which DNA alkylation was detected (24 h, 25 °C).

products (+)-duocarmycin SA and CC-1065 ($10^{-6}$ M, 25 °C), Figure 2. Similar enhancements in the rates of DNA alkylation were also observed. For example, *N*-BOC-CPyI ($10^{-2}$ M) provided complete consumption of w794 DNA within 10−20 min (25 °C) in the presence of 100 equiv $Zn(acac)_2$ while only ~10% reaction was observed after 24 h (25 °C) in the absence of the metal cation, providing a rate enhancement of DNA alkylation ≥1000×. Analogous metal cation enhancements in the rates and efficiency of DNA alkylation for the unnatural enantiomer of **1** were also observed (data not shown). However, similar treatments of **2** and **3** (alkylation at $10^{-2}$ M) or duocarmycin SA and CC-1065 (alkylation at $10^{-6}$ M) did not affect their DNA alkylation rates or efficiencies (Figure S1), indicating this behavior is unique to CPyI (**1**) and its 8-ketoquinoline core structure.

We have interpreted this unique behavior of **1** to represent simply an in situ metal cation complexation and activation for DNA alkylation (Figure 1) analogous to the activation toward $CH_3OH$ addition (Table 1). However, the metal cation could play

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(6) We attribute this behavior of $Zn^{2+}$ relative to $Ni^{2+}$ and $Cu^{2+}$ ($Zn^{2+}$ > $Ni^{2+}$ > $Cu^{2+}$) not to an alteration in the relative activations from that expected ($Cu^{2+}$ > $Ni^{2+}$ > $Zn^{2+}$), but rather to an enhanced selectivity under the reaction conditions employed. That is, the greater activation by $Cu^{2+}$ and $Ni^{2+}$ leads to more nonproductive solvolysis relative to $Zn^{2+}$, lowering the apparent efficiencies of DNA alkylation. For any given application, the optimal results are going to depend on the reaction conditions (solvent, buffer, temperature, time) and the optimal catalyst from the range of metal cation catalysts can be established to tune the reactivity.

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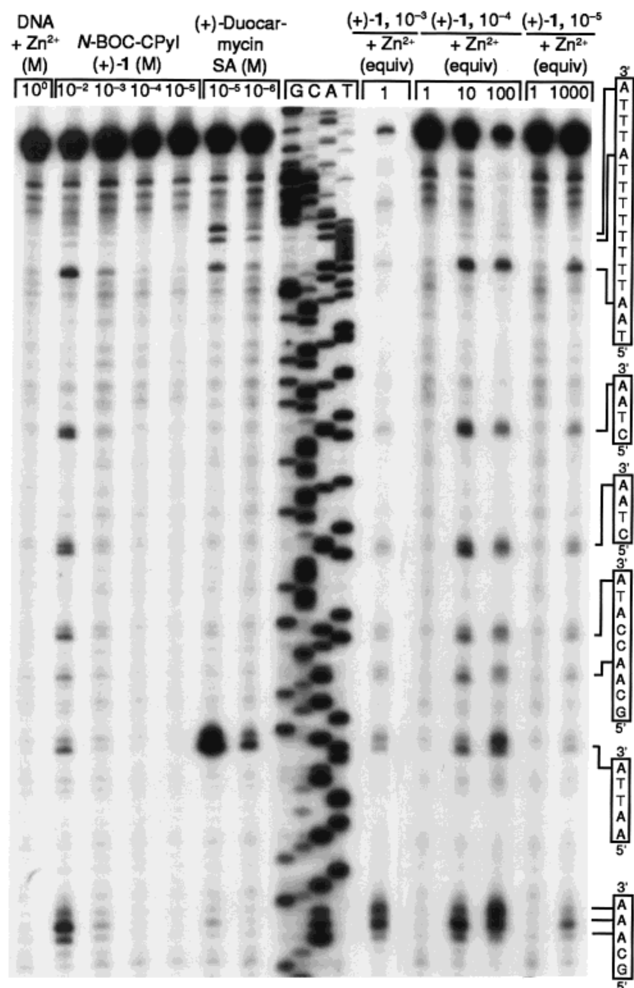


**Figure 2.** Thermally induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138−5238); DNA−agent incubation for 24 h at 25 °C, removal of unbound agent and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography; lane 1, control DNA with Zn(acac)$_2$ (1 M); lanes 2−5, (+)-*N*-BOC-CPyI (**1**, $1 \times 10^{-2}$ to 1 M); lanes 6−7, (+)-duocarmycin SA ($1 \times 10^{-5}$ and $1 \times 10^{-6}$ M); lanes 8−11, Sanger G, C, A, and T sequencing reactions; lane 12, (+)-*N*-BOC-CPyI (**1**, $1 \times 10^{-3}$ M) with Zn(acac)$_2$ (1 equiv); lanes 13−15, (+)-*N*-BOC-CPyI (**1**, $1 \times 10^{-4}$ M) with Zn(acac)$_2$ (1, 10, and 100 equiv); lanes 16−17, (+)-*N*-BOC-CPyI (**1**, $1 \times 10^{-5}$ M) with Zn(acac)$_2$ (1 and 1000 equiv). Note: In lanes containing extensive DNA cleavage, the greater intensity of the shorter oligonucleotide cleavage sites observed at the lower half of the gel is a consequence of multiple cleavages producing greater proportions of the short fragments and do not represent a change in the alkylation selectivity.

a more active role in facilitating the binding of **1** to DNA and its alkylation.[7,8] DNA−metal interactions that increase the affinity of **1** for DNA, or alter the structure or conformation of DNA in a way that enhances its reactivity toward **1** could explain the observations.[8] However, the lack of metal cation effects on the behavior of **2** and **3** and CC-1065 or duocarmycin SA suggests this is unlikely. Similarly, 1:1 or 2:1 complexes of **1** with the metal cations analogous to mithramycin,[9] daunomycin,[10] chromomycin,[11] and quinobenzoxazines[12] may simply provide a higher affinity ternary complex with DNA increasing the apparent efficiency of DNA alkylation. However, unlike these examples, **1** and related agents interact with DNA in an identical manner in the absence of the metal cation, albeit at a reduced rate and with a reduced efficiency, suggesting the primary role of the metal cation is simply catalysis.

In addition to representing a new and tunable method of in situ activation of a novel class of DNA alkylating agents, the observations have implications on the interpretation of the behavior of CC-1065 and the duocarmycins.[13] First, the majority of the efficiency distinctions observed between the simple alkylation subunits such as **1**−**3** and the natural products ($10^3\times$ of the $10^4\times$ difference) may be attributed to ineffective catalysis of the DNA alkylation reaction with **1** and related agents and not their intrinsic capabilities or reversibility[14] (kinetic effect). The remaining 10-fold difference may be attributed to differences in the noncovalent binding affinity or the minor groove positioning and orientation of the agents consistent with identical conclusions drawn from the results of unrelated studies.[15] Our interpretation of this is that **1**−**3** lack the structural features required for catalysis derived from a DNA binding-induced conformational change in the natural products which disrupts the cross-conjugated and stabilizing alkylation subunit vinylogous amide activating them for nucleophilic attack.[16] Second, studies with more advanced CPyI analogues[3] related to the structures of CC-1065 and the duocarmycins, like those with **1** illustrated in Figure 2, have shown that the DNA alkylation selectivity is unaffected by the metal cation catalysis. This indicates that the source of the alkylation selectivity is not uniquely embedded in the catalysis source which is consistent with proposals that it is derived from the compounds' noncovalent binding selectivity.[14,17]

We are unaware of other examples of metal cation initiation of a DNA alkylation reaction, and as such, the studies detailed herein appear to constitute the first example. Intriguingly, comparative trace metal analysis of cancerous and noncancerous human tissues have revealed significant distinctions.[18] Although no generalizations were possible across all tumor types, within a given tumor type these were significant and potentially exploitable differences. For example, Zn was found in breast carcinoma at levels 700% higher than in normal cells of the same type, while lung carcinoma exhibited a reversed and even larger 10-fold difference. Thus, chemotherapeutic agents subject to Zn activation might exhibit an enhanced activity against breast carcinoma attributable to this difference in Zn levels. Such potential therapeutic applications of this class of agents will be the subject of continuing studies which will complement their use as research tools and as models to probe the source of the duocarmycin and CC-1065 DNA alkylation selectivity and catalysis.

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**Supporting Information Available:** Experimental procedures for conducting the DNA alkylation studies of **1** and a gel figure (Figure S1) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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